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Organic Chemical Waste Characterization for Marine Disposal of Black Rock Harbor Dredged Materials

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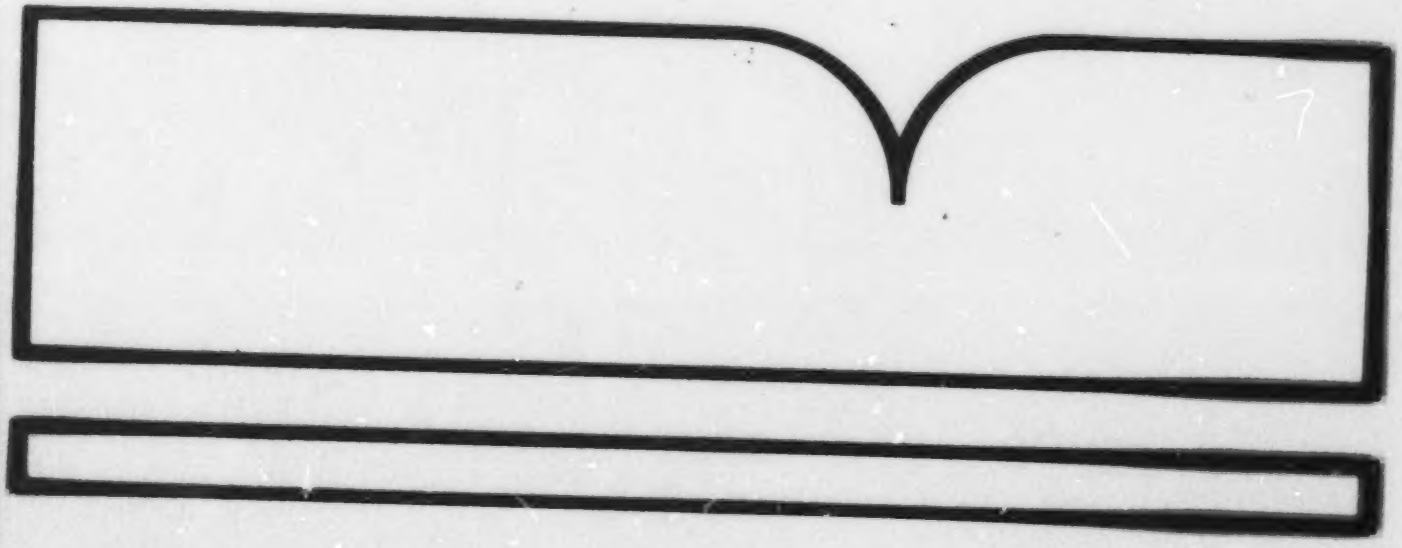

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Organic Chemical Waste Characterization for Marine Disposal of Black Rock Harbor Dredged Materials

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ABSTRACT: A waste characterization test was designed to determine the actual contaminants in dredged materials that are bioavailable. Unlike many smaller freshwater systems, marine habitats are frequently regulated on the basis of waste bioaccumulation potential. This implies that traditional chemical characterization of sediments may provide data that is not appropriate for marine systems. A waste characterization procedure was sought that would determine those contaminants in sediment that could bioaccumulate. A highly contaminated urban harbor sediment from Black Rock Harbor, Bridgeport, CT was chosen as the test material. The marine bivalve, *Mytilus edulis*, was exposed to suspended dredged material for 28 days in a flow-through exposure system. Exposed organisms were then extracted and analyzed for bioavailable, low-polarity contaminants by gas chromatography/mass spectrometry (GC/MS). All separable GC/MS peaks were tentatively identified. These tentatively identified contaminants were subsequently analyzed in the dredged material and quantified when appropriate standards were available. Compounds identified by this waste characterization procedure are assured of being both present in the dredged material and biologically available.

KEY WORDS: waste characterization, sediment characterization, bioaccumulation, bioavailability, gas chromatography/mass spectrometry, qualitative analysis, polynuclear aromatic hydrocarbons, polychlorinated biphenyls, Ethylan, gas chromatography, marine, *Mytilus edulus*, mussels

Bioaccumulation of chemical contaminants from dredged materials has long been a concern to both environmental regulators and the general public. Public Law 92-532, the Marine Protection, Research, and Sanctuaries (the Ocean Dumping) Act, and the regulations arising from it include bioaccumulatable contaminants and bioaccumulation tests as key portions. However, these tests are based on the state of the art in 1977, and may no longer be the best available methodology. Therefore, improvements and changes to these tests should be considered. Although selection and exposure of the marine organisms in the bioaccumulation test could also be improved, this paper deals with the method for selecting which chemicals in a complex waste, such as contaminated sediment, should be studied for their bioaccumulation potential.

Most methods for determining whether a waste contains bioaccumulatable contaminants start with a preselected list of candidate contaminants. An exposure is then conducted, and the exposed organisms are assayed for the selected contaminants. Absence of these specific contam-

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inants at some preselected detection limit is taken as proof that there are no bioaccumulation problems with the waste. However, the composition of discharges into marine systems can be vastly different, and range from industrial and municipal effluents and sludges to both clean and contaminated dredged materials from all areas of our coastline. These different discharges will clearly contain many different contaminants, and make the task of predicting which contaminants could bioaccumulate difficult at best.

The current procedure of preselecting a list of contaminants to be assayed in exposed marine organisms has one of two critical limitations:

(1) either the list is severely restricted to make it manageable, or
(2) it is inclusive and therefore onerously long and expensive.

The first constraint leads to decisions which may not be protective, while the second may not be possible in a cost-effective manner with current analytical methodology.

However, this paper suggests another approach not constrained by these limitations. This approach is to let a marine organism accumulate those contaminants from the complex waste which are bioavailable. A skilled analyst can then identify the bioaccumulated contaminants using qualitative identification by gas chromatography/mass spectrometry (GC/MS) and other appropriate techniques. These identified contaminants then form a waste-specific list of only those contaminants that are biologically available to a marine organism in a bioavailability test. The list is specific, and contains only contaminants that are known to be present in the waste. A limiting corollary is that the list only contains compounds detectable by the extraction and analysis scheme, which has a detection limit of approximately 1 to 10 ng/g.

This approach has been tested as part of the Field Verification Program (FVP), a joint Environmental Protection Agency (EPA) and Corps of Engineers (CE) project to study the field application of laboratory bioassay techniques to a contaminated dredged materials disposal site. The contaminated dredged material site was a small, highly urbanized estuary called Black Rock Harbor (BRH) in Bridgeport, CT. Some 55 000 m^3 of highly contaminated material was dredged from an upper reach of BRH by a clamshell dredge, and barged to the northeast corner of the Central Long Island Sound (CLIS) disposal site (Fig. 1), where it formed a pile about 2 m in height and 200 m in radius. Before dredging, 25 barrels of sediment were collected from the reach by box corer and homogenized. This sediment was used for waste characterization. Contaminants found in this bioaccumulation waste characterization test were then followed in both laboratory and field biological effects testing [*1–6*].

Experimental Procedure

Bioaccumulation waste characterization studies were conducted using *Mytilus edulus* (the blue mussel) as the test organism. *Mytilus* was chosen because it has been extensively used in marine monitoring programs such as Mussel Watch [*7*], the Coastal Environmental Assessment Station (CEAS) program [*8*], and municipal sewage discharge monitoring programs such as the 301(h) program. *Mytilus* exposures were conducted in flowing seawater at 15°C for a period of 28 days with 10 mg/L of added BRH sediment. The mussels were fed a suspension of algae (*Phaeodactylum tricornutum* and *T-Isochrysis galbana*) at 0.5 mg/L to maintain their health. Control *Mytilus* were fed the same algal concentration. The controls acted only as a chemical control to verify that contaminants accumulated by the *Mytilus* came from BRH and not from the exposure system and food.

Samples of mussels were collected at 28 days. Sediment dosing was controlled by a light transmissometer coupled to a dosing valve [*9*]. This allowed the dosing valve to replace sediment that was removed by the *Mytilus* independent of the flow rate through the tank.

Mytilus samples from both BRH exposed and control tanks were removed and frozen before

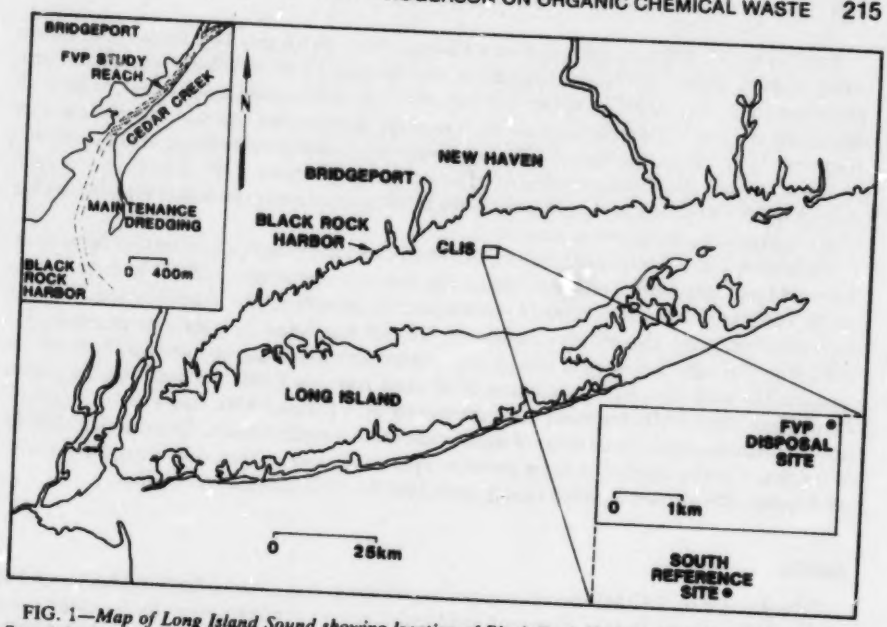

FIG. 1—*Map of Long Island Sound showing location of Black Rock Harbor and Central Long Island Sound dredged material disposal site.*

analysis. Methods of sample preparation and analysis have been described in detail elsewhere [*1*]. Samples of mussels were allowed to thaw and the flesh homogenized with a Polytron®. Initial extraction and dewatering was accomplished with 15 mL of acetone (twice) using a Polytron® with centrifugation to separate the phases. The acetone extracts were combined, diluted with 150 mL of water, and extracted with 50 mL of Freon 113®. Mussel tissue extraction was continued with two 25 mL aliquots of Freon, and the Freon extracts were combined. Preliminary cleanup was accomplished by passing the unconcentrated Freon extracts through an activated (125°C overnight) silicic acid column and eluting with 50 mL of methylene chloride. This removed most interfering biogenic components and allowed reproducible fraction separation in subsequent steps. However, this can remove polar contaminants, but these typically do not bioaccumulate. The cleaned extracts were then concentrated to about 5 mL using a Kuderna-Danish evaporater. Hexane was then added and the concentration continued to remove methylene chloride.

The cleaned extract was then separated into three fractions on a 5% by weight water deactivated silica gel column. Fraction 1 was eluted with 50 mL of pentane, and consisted of aliphatic hydrocarbons, polychlorinated biphenyls (PCBs), and related nonpolar compounds. Fraction 2 was eluted with 35 mL of 20% methylene chloride in pentane (volume/volume) and consisted of polynuclear aromatic hydrocarbons (PAHs) and related slightly polar compounds. Fraction 3 was eluted with 35 mL of 100% methylene chloride and should contain somewhat more polar compounds. The volumes of solvent required to elute fractions 1 and 2 are set so that all PCBs elute in fraction 1, and all PAHs from molecular weight 166 through 302 elute in fraction 2. These volumes must be adjusted for each batch of silicic acid.

Sediment samples were prepared by similar procedures, with the addition of copper powder activated with 8*M* hydrochloric acid (HCl) to the preliminary cleanup column to remove elemental sulfur.

Analysis by GC/MS was conducted on a Finnigan 4500 with a standard Incos® data system using electron impact ionization. Separation was through a J & W DB-5 30-m column programmed from 50 to 330°C at either 4 or 10°/min, with a final hold long enough to elute all resolvable compounds including coronene. The mass spectrometer was scanned continuously from m/z 15 to 550 in one second. The instrument was tuned in accordance with established quality assurance specifications using decafluorotriphenylphosphine [*10*]. Sensitivity was such that 100 pg of PAHs from molecular weight 166 to 252 gave an easily quantifiable molecular ion with a signal-to-noise ratio of at least 50:1.

Qualitative analysis was conducted by GC/MS using a combination of standard Incos spectral matching, relative GC retention times, and spectral interpretation. Since authentic standards were not available for many of the compounds identified, most identifications must be considered tentative. Quantitative analysis by GC/MS was based on integrated molecular ion intensities that were examined to remove any interferences. Appropriate standards were run for quantitation each day, but quantitation of all alkyl homolog PAHs are based on the parent PAH. Since alkyl PAHs fragment more extensively than parent PAHs, this leads to a systematic, but reproducible, low estimate of the amount of these contaminants. Quantitation of PCBs in fraction 1 was by capillary column electron capture GC, and was based on a comparison with an Aroclor 1254 standard, which closely resembled the PCB distribution in BRH.

Results

Total ion current GC/MS chromatograms are shown in Figs. 2 and 3 for fractions 1 and 2 of 28-day BRH exposed *Mytilus*. Figures 4 and 5 reveal similar chromatograms for fractions 1 and 2 for the control 28-day *Mytilus*. These traces reveal that many contaminants present in BRH were biologically available to *Mytilus* and did bioaccumulate. The presence of distinct peaks in

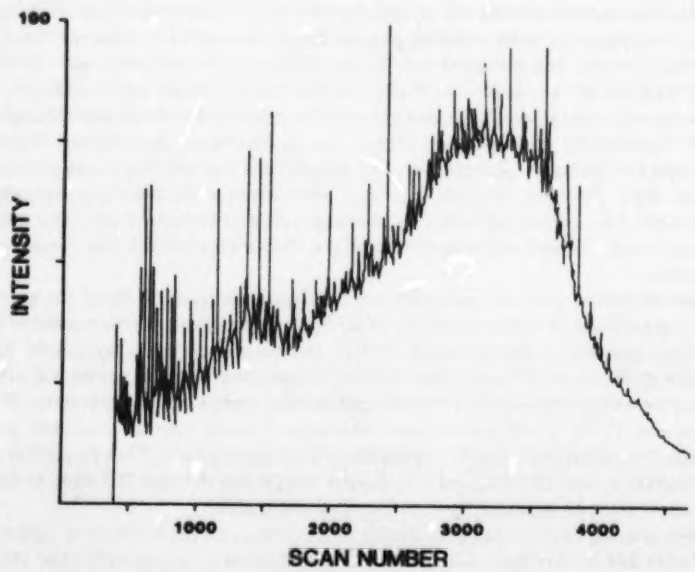

FIG. 2—*Total ion current chromatogram of fraction 1 (PCBs and saturated hydrocarbons) from BRH exposed mussels,* Mytilus edulus.

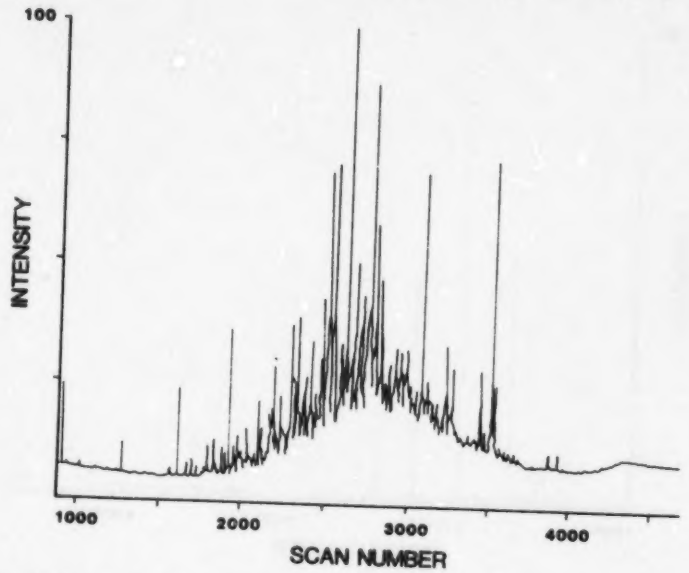

FIG. 3—*Total ion current chromatogram of fraction 2 (polynuclear aromatic hydrocarbons) from BRH exposed mussels,* Mytilus edulus.

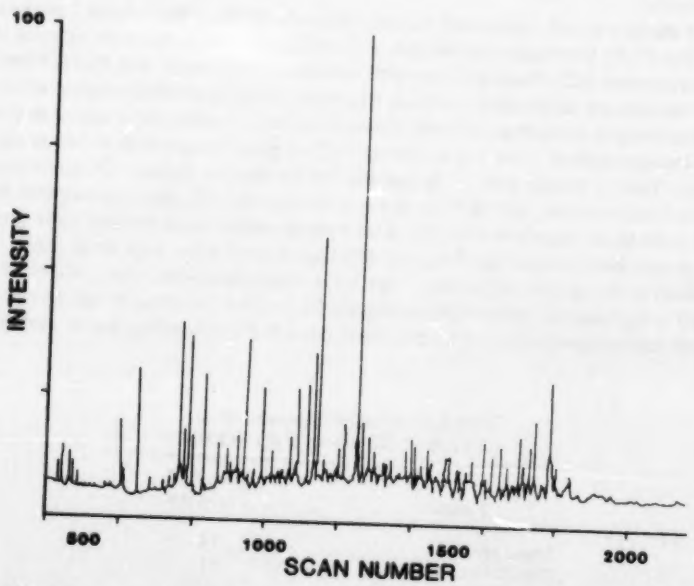

FIG. 4—*Total ion current chromatogram of fraction 1 (PCBs and saturated hydrocarbons) from control mussels,* Mytilus edulus.

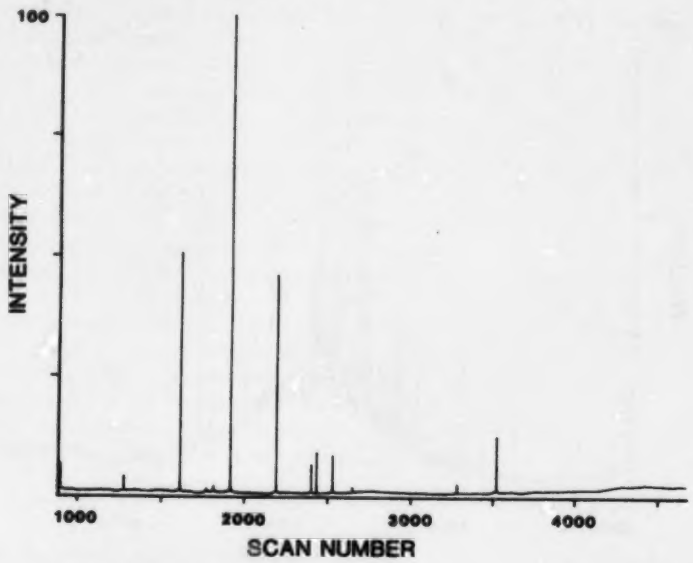

FIG. 5—*Total ion current chromatogram of fraction 2 (polynuclear aromatic hydrocarbons) from control mussels,* Mytilus edulus.

Figs. 4 and 5 shows that there were also several contaminants or biogenic compounds present in control *Mytilus*.

Figure 2 shows a broad, unresolved complex mixture (UCM) from fraction 1 of the exposed mussels. This UCM is typically seen in both sediments and bivalve organisms exposed to petroleum contamination [*11*]. Fraction 1 contains alkanes, cycloalkanes, and PCBs. Mass spectra from this fraction are dominated by alkane fragments at low molecular weights, which makes spectral matching of unknowns difficult. However, at higher molecular weights, several peaks could be distinguished as belonging to various PCB congener classes of from two to eight chlorine atoms. Table 1 reveals total PCB quantitation by electron capture GC as Aroclor 1254. Control concentrations are similar to those reported elsewhere [*7*]. Also present were several of the DDT series in low concentration, but these were of similar concentration in the control and exposed groups and so were not pursued. Although several other high mass peaks could be distinguished in the spectra of fraction 1, no other compounds of interest could be identified.

Figure 3 is the total ion current chromatogram for fraction 2 of the exposed *Mytilus*. It contains many resolved peaks that can be tentatively identified by a combination of spectral match-

TABLE 1—*Total PCB concentrations (as Aroclor® 1254) in ng/g dry weight.*

Sample	Total PCBs, ng/g dry
Day 0 *Mytilus*	74
Day 28 Control *Mytilus*	84
Day 28 Exposed *Mytilus*	3000
BRH Sediment	68000

ing, relative retention times, and spectral interpretation. The vast majority of peaks corresponded to PAHs, both the parent compounds and their alkyl-substituted homologs. Electron impact mass spectrometry is not capable of resolving between different isomers of these compounds, but most parent PAH isomers can be distinguished by their relative GC retention times on the DB-5 column. No identification was attempted for alkyl homolog isomers beyond the parent molecular weight and the alkyl carbon group. For example, all methyl anthracenes and phenanthrenes were combined in a (C-1) 178 class, because they have a single alkyl carbon on a parent molecular weight 178 PAH. Tables 2 and 3 list PAH compounds and isomer classes found in both control and BRH exposed *Mytilus*.

These parent and alkyl homolog PAHs form the bulk of compounds discernable in fraction 2, but are not the only compounds present. At approximately scan 3500, there is a large peak next to the smaller benzo(e)pyrene, which corresponds to the isoprenoid hydrocarbon squalene, a common biogenic hydrocarbon in marine systems. Another unusual peak was detected at scan 2801, which appeared to be to a nitrogen-containing aromatic hydrocarbon with a molecular weight of 223. However, spectral matching and injection of an authentic standard revealed that this mass corresponded to a fragment of the pesticide Ethylan, a DDT analog in which the aromatic chlorine atoms have been replaced with ethyl groups. There are three isomers of Ethylan that may correspond to *o,o-*, *o,p-*, and *p,p-*. Near the beginning of the chromatogram are a series of evenly-spaced large peaks, which have been tentatively identified as siloxanes of

TABLE 2—*PAH concentrations (ng/g dry) in exposed and control* Mytilus *(± standard deviation, n = 3) and in Black Rock Harbor sediment.*

Compound	Molecular Weight	Day 0 Control	Day 28 Exposed	Day 28 Control	BRH Sediment
Fluorene	166	1.4 ± 0.4	12 ± 3.7	1.2 ± 0.4	370
Phenanthrene + anthracene	178	7.2 ± 1.6	180 ± 35	6.7 ± 1.5	2700
Pyrene + fluoranthene	202	34 ± 8	2000 ± 470	19 ± 2.5	7100
Chrysene + triphenylene + benz(a)anthracene	228	8.5 ± 2.3	1800 ± 360	3.1 ± 1.1	9800
Perylene + benzopyrenes + benzofluoranthenes	252	15 ± 5	1600 ± 210	5.8 ± 1.7	8600
Benzoperylene + dibenzopyrene + others	276	3.3 ± 0.7	280 ± 50	1.6 ± 0.9	9100
Benzochrysenes + dibenzanthracenes + others	278	<0.4	160 ± 20	<0.6	4400
Coronene	300	<0.1	9.3 ± 0.8	<0.1	130
Dibenzochrysenes + others	302	<0.3	56 ± 9	<0.1	2700

TABLE 3—*Sums of PAH alkyl homolog concentrations (ng/g dry) from C-1 to C-4 substituent carbons in exposed and control* Mytilus *(± standard deviation, n = 3) and in Black Rock Harbor sediment.*

Alkyl-PAH Compound[a]	Day 0	Day 28 Exposed	Day 28 Control	BRH Sediment
166[a]	14 ± 3.4	1100 ± 250	23 ± 4.8	3400
178[a]	38 ± 6.7	5000 ± 1400	39 ± 7.1	13 000
202[a]	12 ± 1.9	3400 ± 690	7.7 ± 2.9	8300
228[a]	5.9 ± 1.6	1800 ± 290	1.3 ± 0.6	13 000
252[a]	<6	590 ± 79	<5	4800

[a]Molecular weight of parent PAH for which alkyl homolog sums of 1 to 4 carbon substituents were quantified.

different chain lengths and isomers. The three most evident ones are at scan 1277, 1619, and 1927. Although both the isotope ratios of silicon and their fragmentation patterns are consistent with this identification, no further verification was attempted because of the large number of possible isomers of these compounds.

Control *Mytilus* total ion current chromatograms are shown in Figs. 4 and 5 for both fractions 1 and 2. Figure 4 shows little of the UCM from petroleum contamination evident in Fig. 2, but there are small peaks from DDTs and PCBs. Most other peaks are either alkane-type spectra or other biogenic hydrocarbons. Figure 4, on the other hand, contains several very large peaks, which have the same spectra and retention times as the tentatively identified siloxanes in the BRH exposed mussels. Also evident on Fig. 4 is the biogenic hydrocarbon squalene around scan 3500. Very few PAHs and no Ethylan were detected. Table 4 lists the retention times and the areas of these siloxane peaks normalized to a dry weight basis for comparison of control and exposed *Mytilus* as well as BRH sediment.

Analysis of BRH sediment revealed higher concentrations of all contaminant compounds than were found in the mussels with the exception of the siloxanes, which were present in much lower concentrations. Since Ethylan was an unexpected contaminant, no authentic quantitation standard was available for this compound.

Discussion

Using a bioaccumulation test in the waste characterization phase of a dredged materials disposal study generates results consistent with the octanol-water partition coefficients (Kow) versus the bioaccumulation potential models of Veith et al. [*12*]. This model predicts that compounds with a high partition coefficient are likely to bioaccumulate. Generally, those classes of compounds were determined that are typically present in contaminated sediment, PCBs and PAHs, and these contaminants were biologically available to a marine bivalve. In addition, an unexpected contaminant, Ethylan, was detected. This contaminant would not have been recognized using most of the preselected lists of analytes. Also, because of the chemical control conducted with the bioaccumulation study, another series of contaminants, the siloxanes, were shown to be a system contaminant because they were present in high concentration in the controls and not present to a significant degree in the sediment. Probable sources of this contamination in the bioassay system include silicone tubing and Silastic® sealant, both of which are frequently used in marine bioassay systems.

The FVP contains both a laboratory and a field component, and the field component contains an exposure assessment component, in which the distribution of contaminants around the disposal site was followed for 1 year predisposal and 3 years postdisposal [*13*]. This allowed ample opportunity to gather chemical contaminant concentrations in various environmental compartments around the disposal site for comparison with those contaminants that were deter-

TABLE 4—*Silicone-like concentrations in GC/MS counts/gram dry weight in exposed and control* Mytilus *(± standard deviation, n = 3) and in Black Rock Harbor sediment.*

GC/MS Scan Number	Day 0	Day 28 Exposed	Day 28 Control	BRH Sediment
1277[a]	...	23 000 ± 5900	28 000 ± 2600	1800
1619[a]	...	48 000 ± 17 000	550 000 ± 120 000	1500
1927[a]	...	86 000 ± 39 000	1 140 000 ± 150 000	480
2194[a]	...	45 000 ± 21 000	412 000 ± 73 000	480
2432[a]	...	10 000 ± 4100	53 000 ± 13 000	330

[a]GC/MS scan number, in seconds into the GC run.

mined to be both present and available in the dredged material. Although not specifically designed to test the waste characterization procedure, this exposure assessment effort provided good information on the validity of the approach. Periodically, samples of water, sediment, and organisms were collected and analyzed from around the disposal site. All of these samples were analyzed by GC/MS and no additional contaminants were discovered.

Most contaminants were present in most samples over the 3-year postdisposal period. A significant exception was Ethylan, which disappeared from the area more quickly than other contaminants and was usually not found in the reference site. Also, not all PAHs were found in all water samples. With these exceptions, most contaminants were detected in most samples. This included GC/MS analysis of all fraction 2 samples for PAHs and Ethylan. Throughout this study, no additional contaminants were detected that were associated with the dredged material. This is not to say that all the contaminants in the sediment have been determined. For example, there are probably a series of volatile contaminants such as solvents present, and many contaminants that will not pass through a gas chromatograph. In addition, because the same extraction and analysis schemes were used throughout the study, contaminants that were beyond the analytical scope of the waste characterization would have remained beyond the scope of the investigation.

Within the scope of these limitations, the waste characterization method allowed analytical effort to be focused on those compounds that were actually present and likely to be available to marine organisms. Although there was excellent agreement between those contaminants found in the waste characterization and those found at the disposal site, the method provided no information on the extreme variability of contaminant concentrations found in the actual disposal site. Repeated analysis of the barreled, homogenized BRH sediment revealed relative standard deviations for most contaminants of about 25%. By comparison, adjacent samples from the center of the disposal mound could differ by a factor of five for PAH concentrations. Cores through the pile revealed clear bands of clay and contaminated sediment with contaminant levels orders of magnitude apart. This heterogeneity of the disposed material may be a result of the particular dredging method used (a clamshell dredge), which results in some coherent pieces of sediment being moved. Although this was a maintenance dredging operation, the indication is that some underlying material was probably removed along with the recently deposited contaminated sediment. This extreme heterogeneity underscores the necessity for extensive replicate sampling in the field.

Conclusions

A waste characterization procedure for marine dredged materials has been developed. It is based on determining which contaminants in a dredged material are biologically available and will accumulate in the bivalve mollusc *Mytilus edulus*. A major advance is that this test eliminates contaminants from consideration that are either not present in the material or not likely to be biologically available. Contaminants determined with this procedure have a high probability of being the compounds of interest in monitoring studies around disposal sites in the field. By focusing scarce analytical resources on only those contaminants both present and available, greater effort can be spent on detecting those identified contaminants at or near ambient concentrations so that small elevations above background can be determined.

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